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EDITORIAL

CONTROL OF DISEASE BY VACCINATION

No doubt can exist on the value of vaccination in the control of infectious disease. There is ample evidence in many parts of the world of the enormous benefits derived from the active immunisation of livestock: in fact, it is not too much to claim that without such a method of control it would be well nigh impossible to raise livestock successfully in some areas, and that the economy and even the life of such areas would be seriously imperilled. The control of rinderpest is a classical example, and the responsible authorities must receive much credit for the introduction of vaccination as a method of control. The type of vaccine used must always receive careful consideration. As research work progresses and more workers are engaged on the study of immunity and immunisation, new ideas on types of vaccine are forthcoming, and the type used in the field eventually is changed. Again, rinderpest vaccination is a good example to quote. The evolving of the more modern vaccines has taken place as more general scientific information has become available: as more knowledge of viruses, their cultivation, attenuation and preservation has been made available, so have the types of vaccines been altered and used in the field.

It is sometimes difficult to come to a conclusion regarding the advisability of introducing vaccination into a country as a method of control of a disease. Many considerations have to be taken into account: but it would appear to be sound reasoning that if a disease is not endemic in a country, a "stamping out" method should be applied rather than control by vaccination. The "slaughter policy" practised in dealing with foot and mouth disease in this country has successfully prevented the disease from becoming endemic. A "slaughter policy" can, of course, only be successfully applied if early notification of suspected disease is given and prompt action is taken by the responsible authority. There is always a danger in the absence of a disease from a country for a prolonged period that suspicions of its presence may not receive the essential immediate attention of stock-owners and others. Those who are in a

position to impress the need for full collaboration in the early reporting of the suspected disease are doing a real service by impressing upon livestock owners the vital importance of early action. In a country such as this, unless the foot-and-mouth disease position became very different it is extremely unlikely that vaccination would be considered. In other countries, however, in which the disease has become endemic, there is every reason why vaccination should be adopted as the method of control, and, with the use of modern types of vaccine, reports and records would indicate that beneficial results are obtained. Our experiences of vaccination in this country for the prevention of brucellosis in cattle show quite clearly that provided the recognised type of vaccine is used under conditions that have been carefully worked out, the incidence of the infection can be materially reduced and, in some herds, totally prevented. The best results following the use of any vaccine, however, can be expected only if the prescribed method is carried out. With live vaccines, for example, care has to be taken to place a " life " on the product, during which it may be used. Much research work and thought, followed by laboratory and field experiments and trials, have taken place before the recommendations concerning the use of a vaccine are made.

Experimentation is continually going on. In this issue of the BRITISH VETERINARY JOURNAL we publish an account of research work on vaccination against fowl pest (Newcastle disease). It has been claimed in most countries that in order to obtain satisfactory immunity against the causal virus the vaccine must contain living virus, which may, of course, be in an attenuated state. The work now recorded would indicate that a satisfactory immunity may be set up by the use of a vaccine in which the virus is apparently killed. The successful results following the use of vaccine in the control of swine fever, in which the virus is so treated that it is probably killed, no doubt lead to the investigation of a similar method of vaccine production for use against fowl pest (Newcastle disease). There must always be an advantage in using such a vaccine, and if further experiences show that the results of the reported investigations can successfully be applied in the field, we will have in our hands yet another efficient product, incapable of causing harmful results but capable of setting up an immunity of high order. Much credit must go to research workers in the laboratory and in the field who patiently carry out the essential experiments which lead to the evolving of these products, the use of which are attended with such highly successful results.

GENERAL ARTICLES

THE ANIMAL HEALTH TRUST

By W. R. WOOLDRIDGE, Ph.D., M.Sc., M.R.C.V.S.,
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The Origin of the Trust

FOUR personal experiences may fairly be said to have led to the foundation of the Trust. The first occurred at Cambridge, when the writer was a junior member of a research staff, wishing to obtain a research fellowship, and finding that not a single one existed in the veterinary field. Although an application for one of the many medical fellowships happened to prove successful, the idea of a veterinary trust was born, together with the determination—should the chance of persuading someone to endow a trust ever come about—to provide the veterinary profession with the same post-graduate opportunities as those enjoyed by people taking human medicine or the natural sciences.

The next relevant experience was with the Loveday Committee on Veterinary Education in Great Britain, through which a great deal of information was acquired about the facilities for veterinary education, and, to a lesser extent, veterinary research in this island. That Committee visited all the Colleges here and some of those on the Continent of Europe. The writer was shocked to find that the facilities available then in some of the smaller countries abroad far exceeded those available in this country.

The third experience consisted of some fifteen years of research and teaching in world-renowned scientific and medical institutions, where the writer was in close association with research men in various branches of science, and was privileged to work with such scientific leaders as Sir Frederick Gowland Hopkins, Sir William Hardy and Professor Topley. While all were vaguely interested in the welfare of the veterinary profession, and wished to see it develop, most of these scientists were curiously ignorant of the rapid progress being made by the profession under the greatest possible difficulties, and of the high standard of work which had already been achieved. It was clear that something had to develop within the profession to establish it firmly on the "scientific map."

The fourth personal experience was that of helping, during the war, the National A.R.P. for Animals Service, when the discovery was made of the comparative ease with which various societies were able—provided they knew how to organise and launch an appeal—to raise large sums of money from the public for animal welfare work.

It was during the writer's Presidency of the N.V.M.A., when progress in veterinary education and research seemed to have come to a halt, that these four experiences came forcibly to mind. An unofficial approach was made to the Ministry of Agriculture, to ascertain whether they intended implementing any of the recommendations of the Loveday Committee, insofar as the facilities for the veterinary schools were concerned. The answer received was that nothing was being done until the war was over. This, together with the war-time

difficulties being experienced by the Colleges, emphasised the need for action. Although there was no rich benefactor in the offing, the idea of founding a trust took shape. Steps were taken to contact a number of influential people who, it was thought, might prove sympathetic, and it was encouraging to find that only one of those approached did not welcome the idea or offer full support.

That was the beginning of the Trust. In some ways it was an unfortunate beginning, in that its birth—announced at a Mansion House luncheon in September, 1942—had to be kept secret, in view of its apparently "impossible" aims, until the last moment, and thus came as somewhat of a bomb-shell to the profession. Warmly welcomed by the Minister of Agriculture and others, the Trust began its career under the auspices of many famous men, but with practically no money. Obviously, it had to begin in a small way, and for this reason the full extent of its objects, as set out in the Trust Deed, was not at first published. For a new organisation, without funds, to have done so would have been to risk disaster.

Development of the Trust

The title of Veterinary Educational Trust had been chosen in order to facilitate the organisation being accepted within the category of an educational trust as opposed to other charities having no educational aims. It was on this side of the Trust's work that a beginning was made. Undergraduate scholarships were offered to the value of £200 for a period of five years. It was thought that these would enable the student, freed from financial worries, to derive the fullest benefit from his training. Later, this type of scholarship was abandoned, partly owing to the high proportion of ex-Service students necessarily accepted by the Colleges, which restricted normal entry and made fair awards difficult or impossible; and partly because many requests for assistance began to come in from students who had begun their courses and then, through adverse circumstances, found themselves unable to complete them. Scholarships have now, therefore, been restricted almost entirely to students coming into this latter category.

The Trust's next step was to offer certain specific research fellowships. These were intended to try to encourage students or young graduates to embark upon a career of research or specialisation. Some fellowships were for specified branches of work; others were kept completely untied, so that a young graduate could choose his own particular line and stand a chance of securing one of these fellowships. Their value varied from £450-£800 per annum, and they have been granted, as a rule, for a period of three years, subject to annual reports of satisfactory progress. It should be added that fellowships have been the means of some graduates gaining experience abroad, and that post-graduate work in surgery, medicine, and clinical subjects generally, has been catered for in addition to purely laboratory studies. Besides assisting some British graduates to go abroad, the aim has been to effect some degree of inter-change: graduates from overseas visiting the Trust's research stations. The Fison Lectures were established to induce an expert in some field of veterinary work to give one or

more lectures on work of importance to the profession. Thus, in 1948, Professor Seekles came here from Utrecht to lecture on the influence of feeding upon animal health.

That, briefly, was the first stage in the development of the Trust; apart, of course, from the raising of the necessary money. The latter was the subject of some unforeseen difficulty, for it had not been appreciated that the Agricultural Red Cross appeal would preclude the Trust from launching an appeal of its own. Despite this initial handicap, however, the Trust has succeeded in raising a reasonable sum, which is increasing year by year. In these early years it did, indeed, manage to develop its work and at the same time it was able to invest approximately £90,000, which formed a useful nucleus for subsequent borrowing from the bank.

The next stage in the Trust's development concerns the research stations. Between the wars there were many young veterinarians who began to specialise, only to find that the opportunities of entry into a veterinary institute, to continue work in their chosen specialist line, were non-existent. Such people were forced either to abandon their particular subject and go into practice, or else enter some service, such as that of the Ministry of Agriculture. These other services benefited, no doubt, from such acquisitions, but the fact remains that the profession lost a number of research workers who would have advanced knowledge if only conditions had been more favourable for their employment. Accordingly, the Trust let it be known that specialist research stations were to be set up, so that if a man wished to specialise in work connected with dogs, horses, poultry, dairy cattle, or some other species of domestic animal, there would be at least some posts available in this country which he might reasonably expect to have a chance of securing.

The Equine Research Station, at Newmarket, was the first to be established. The site was to some extent fortuitous, in that individuals prominent in the bloodstock world placed two estates at the disposal of the Trust under favourable financial conditions. The Canine Research Station was the next to be developed, on an adjoining site. Then came the acquisition of about 900 acres of land upon which to form a group of Farm Livestock Research Stations. Of these, the Poultry Research Station was organised and equipped during 1949, and a start made with the Bovine Research Station.

This second stage in the Trust's development—the setting up of research stations—naturally called for a great deal of popular appeal work in order to raise the necessary funds. And by the beginning of 1948 it was apparent that the name Veterinary Educational Trust was in itself something of a hindrance. It was difficult to convince leaders of agriculture, farming and other livestock interests that this was a Trust to help them, and not merely a Trust to help train individual veterinary surgeons who would later charge them fees. Accordingly, in order to save lengthy and repeated explanations, and to conform with its broadening activities, the organisation was renamed the Animal Health Trust in February, 1948.

The Trust's Educational Work

Up to June 30, 1949, the Trust had spent more than £42,000 on educational work, and had made the following awards:—

Undergraduate scholarships and studentships	41
Evans Final Year Studentships	29
Research Training Scholarships	16
Research Fellowships	24

The work of eleven of the Research Fellows is, or was, concerned with the problems of farm livestock (cattle, 7; sheep, 3; pigs, 1), whilst five dealt with equine and three with canine problems; one with studies concerning wild animals in captivity; and four with more fundamental work. Fellows have been enabled to undertake post-graduate work at, among other centres:—

The Zoological Laboratories, Cambridge;
The Biochemistry Laboratories, Oxford;
The School of Agriculture, Cambridge;
Institute of Animal Pathology, Cambridge;
The London School of Hygiene & Tropical Medicine, London;
University of Wisconsin, U.S.A.;
The Rockefeller Institute, U.S.A.;
The Veterinary Laboratory, Weybridge;
Various Laboratories, University of Liverpool;
The University of Kentucky, U.S.A.;
The Mayo Clinic, Minnesota, U.S.A.

The store set by these fellowships may, perhaps, be indicated by the following extract from a letter received from a Fellow at the termination of his award: "I feel that, rather than the results I have obtained, the post-graduate degree I have gained, or the techniques I have learned, it is the 'scientific outlook' I have acquired which will be of great value in investigating veterinary problems. For this I am particularly grateful to the Animal Health Trust."

A new and important development in the Trust's educational programme is the organisation of short courses of practical and theoretical instruction for those who have the intimate care of animals. So far these activities have been restricted to the running of three courses for studsmen, but it is hoped to arrange in the future similar courses for herdsmen, shepherds, kennel staff and others.

The Trust's educational aims also embrace a wider use of veterinary films, the issue of informative leaflets, and educational exhibits at shows.

The Nature of the Trust's Research Programme

Being an independent body, which receives no financial aid from the State, the Trust is able to pursue an independent research policy. This has many advantages. The Third Report of the Select Committee on Estimates (No. 132 of 1947) stated: "Your Committee are disturbed by the impression they have gained that the conditions of Government service do not always create an atmosphere attractive to research workers or conducive to the achievement of scientific progress . . . . Certain cherished freedoms are lost. Departmental

control tends to have a deadening effect." Again, Sir Patrick Laird, Secretary, The Scottish Department of Agriculture, said last year: "It would be extremely regrettable if the whole burden of supporting work on animal diseases research were to fall upon the State. With the burden always went the calling of the tune. If it were to fall on the State it would quite obviously greatly hamper the spirit of free enterprise and of scientific enquiry." Dr. Slater, Secretary of the Agricultural Research Council, also recently said: "Subject to certain limitations the Council hoped to be able to help and stimulate private enterprise in research, since they were fully alive to its value."

Although there is a certain healthy overlapping, the Trust is being careful to avoid duplication of the research being carried out at Weybridge, Compton and Moredun. In agreement with the Agricultural Research Council, the Trust is concentrating on the diseases of young animals, nutrition, and infertility problems—subjects not, in the main, being investigated at "official" research establishments. Leukosis in poultry, lukæmia in dogs, and neoplasms in most animals are also to be studied by the Trust.

Mr. Tom Williams, Minister of Agriculture, put the position in a nut-shell when he said, in a speech on November 29, 1949: "This work is in no sense a duplication of the work being done under the auspices of the Government. Rather do I regard it as being supplementary to the work being carried out at Government experimental stations. I am sure there is a wide set of problems that is not covered by official stations, and in these the Trust is able to play an important part."

Work at the Trust's Research Stations.

Equine Research Station. A clinical investigation of the causes of infertility in the mare has been continuing for some time, and during the 1949 covering season a total of 60 mares were examined and treated. The previous year's results show that of the mares which were sent by veterinary surgeons for treatment, 66 per cent proved in foal, although a number of them had been barren during the preceding three years. Work on the duration of the fertilising capacity of semen was also begun in 1948, and the information gathered on these two subjects will shortly be available in Progress Report No. 2 (Equine Research Station). Work on ascarid infestation[1] in the foal continues, and a survey of foal diseases has been started. Through the co-operation of neighbouring practitioners it has been possible to carry out a post-mortem examination of every foal which died, other than from dystokia or accidental causes, in the Newmarket district. To date, some 57 newly born foals have been examined, of which about 15 per cent died as a result of infection with *B. viscosum equi,* and a good deal of work has been done with regard to this organism.

The work begun by Parry, in conjunction with veterinary surgeons practising in the Newmarket area and with the School of Pathology at Cambridge, on hæmolytic[2, 3] disease in foals has been continued.

The physiological effects of inductotherm therapy are being investigated, with a view to putting on a less empirical basis the treatment of sprains and strained tendons by this method.

XUM

Canine Research Station. The estate at Kennett did not become available for use until February, 1949, and the conversion of existing buildings and the fitting up of temporary laboratories occupied several months. For this reason, and on account of the absence of adequate animal accommodation, experimental work has been severely limited. Progressive retinal atrophy[4] was the subject of clinical investigation during 1949.

Poultry Research Station. Last July the staff of this station, which is near St. Ives, Huntingdon, were able to move out of their temporary hutted accommodation into well-equipped, prefabricated laboratories. The station has a breeding flock of 300 birds, and some 1,500 pullets undergoing progeny tests. The latter are being carried out mainly in an attempt to establish and study families resistant or susceptible to disease, with particular reference to fowl paralysis and diseases of the leukosis group.

The work of the bacteriology department is mainly concerned with the study of egg-borne diseases, including B.W.D. and other salmonella[5] infections. Investigations in progress include studies in incubator and hatchery hygiene, the possibility of immunising eggs by vaccinating the mother, and respiratory disease. A survey of the incidence of salmonellosis in wild animals is being made, and a routine salmonella service has been established. Over 300 suspected salmonella cultures have been received, from places in England, Wales, Scotland and Northern Ireland, for examination and typing. Salmonella antigens and specific antisera have been supplied to the Veterinary Laboratory at Weybridge and to Veterinary Investigation Officers and others.

In the parasitology department work on the control of coccidiosis[6] by means of sulphapyrazine, sulphaquinoxaline, and other agents, continues. Attention is also being paid to the effects of various methods of management —the deep litter system and heated floors—on the control of the disease. An outbreak of moniliasis[7] in turkeys was investigated during 1949, and work on blackhead is in progress.

Independent Research under Trust auspices. Trichomoniasis was studied by a Research Fellow at Wisconsin University. He was successful in culturing the parasite in developing chick embryos. At Liverpool University, another Research Fellow examined one aspect of the part played by vitamin A in the lactating cow, and a third is working on acetonæmia. A survey to determine the extent to which dogs and cats may harbour salmonellæ was carried out at the London School of Hygiene and Tropical Medicine. Rickets in sheep, and genotype environment interaction in mice, are among other subjects being studied under Trust auspices.

Clearly it is not possible to detail all the work done under Trust auspices, but short accounts of it are given in the three reports so far issued.[8]

County Organisation and Finance

The income of the Trust continues to expand and has now reached over £72,000. This sum included, in 1949, over £53,000 by way of donations, £11,513 in the form of covenanted subscriptions, and over £5,500 as specific

educational grants. Whilst the income has risen, the expenditure incurred by the Trust has very materially increased. During the last financial year £8,200 was spent on veterinary education. The expenditure on research rose to £35,604, this figure being double that for the preceding twelve months. In order to help raise the large sums required for the running of the research stations, and the fulfilment of the Trust's aims, county committees have been formed. There are now eleven of these, and two more will shortly come into being.

The development of county committees led to the decision in 1949 to institute membership of the Trust. Membership falls into four categories—Animal Health Fellows, Animal Health Members, Associate Members, and Junior Members—with appropriate minimum subscription rates, viz. (respectively), ten guineas, one guinea, from five shillings to one guinea, and five shillings. Every effort is being made to establish a strong nucleus of individual members, not merely in the United Kingdom, but throughout the world. Already much interest in the Trust's Animal Health campaign has been evinced in the Dominions and foreign countries, and in many cases that interest has been expressed in terms of generous financial support. In seeking to secure a large membership, the Trust's main purpose is to strengthen the team-work which is essential to the success of its work—the team-work of stock-owners, research workers and practising veterinarians.

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AN INACTIVATED VACCINE AGAINST NEWCASTLE DISEASE

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THE widespread and rapid dissemination of Newcastle disease during recent years in Europe and America has directed attention to the urgent need of finding a method of immunisation against it. Considerable progress has already been made in controlling it in India, Palestine and America by the use of living, avirulent virus vaccines, and the results have, in general, been satisfactory. This type of vaccine confers a solid and probably life immunity, and its wide application in countries in which the incidence of the disease is high is at present the quickest and most economical method of reducing losses and controlling the infection. But the problem presented by the disease in Britain

is different from that elsewhere, as this country, having no land frontiers, is favourably situated for the eradication of the major diseases of animals, and this procedure has, when practicable, always been preferred to large-scale immunisation. In these circumstances the use of living virus would be contrary to established policy, and as there would be grave danger of it perpetuating the infection it would be unlikely to provide an economical solution to the problem. While eradication is undoubtedly the soundest and cheapest policy to pursue in Britain, circumstances might arise in the future in which vaccination could prove a useful adjunct to the existing system of control. But a vaccine, to be suitable, must not contain living virus; it would need to be safe, cheap and capable in a single dose of conferring protection for a reasonable period of time.

Iyer and Dobson (1941) prepared an inactivated vaccine from a tissue-free solution of virus, obtained from chick embryos, to which was added dibasic sodium phosphate and crystal violet, but it gave irregular and unsatisfactory results. Acevedo and Mendoza (1947) used a vaccine prepared from chick embryos, glycerinated buffer solution, crystal violet, sodium borate, and 0.1 per cent formalin; the mixture was stored for ten days in the ice-box before being used. This vaccine in laboratory tests protected fowls against 100 M.L.D. of virus. It gave good results also in the field, but the duration of the protection conferred does not appear to have been determined. According to Coronel (1948), the protection lasted only a short time, and different batches of vaccine varied in potency. A considerable amount of work has been reported from various countries on the preparation of inactivated vaccines against this disease, and although many of these have conferred good protection, the duration of the protection has invariably been too short to be of much value under field conditions.

In work (not yet published) carried out some time ago at this Institute, an inactivated crystal violet tissue vaccine was prepared against swine fever. This vaccine conferred a solid immunity for at least 12 months, which is similar to that conferred by crystal violet blood vaccine against swine fever. In view of this result, it was decided to test the value of a vaccine prepared from chick embryo tissue against Newcastle disease.

Tests of Inactivated Vaccine

Preliminary tests were made on fowls and pigeons with three different vaccines.

Vaccine I: "Herts" virus. Chick embryos and allantoic fluid.
Vaccine II: "Herts" virus. Egg yolks only
Vaccine III: "Turkey" virus. Contents of eggs minus albumen.

The fowls and pigeons were tested for immunity by the inoculation of 1 c.c. virus* (1 : 50 allantoic fluid) 23 days after vaccination; the homologous virus was used in each test. (Table I.)

*The M.L.D. of neither the "Herts" nor the "Turkey" allantoic fluid had been estimated before use in this experiment, but from subsequent titrations on four-month-old chickens it was found to be 10^{-8} for "Herts" and 10^{-6} for "Turkey," so the test dose of both viruses was unduly severe.

TABLE I.

Vaccine	Bird	Dose of vaccine c.c.	Results
I	F.1886	0.5	Remained normal.
	F.1867	1.0	Killed in extremis—7th day.
	F.1868	1.0	Remained normal.
	F.1869	2.0	" "
	P.1	1.0	" "
	P.2	1.0	" "
	P.3	1.0	" "
	P.4	1.0	" "
II	F.1877	0.5	Severe reaction—recovered.
	F.1876	1.0	Remained normal.
	F.1875	1.0	" "
	F.1874	2.0	" "
	P.5	1.0	" "
	P.6	1.0	" "
	P.7	1.0	" "
	P.8	1.0	" "
	F.1895	Control	Died 6th day.
	F.1896	"	Died 8th day.
	P.9	"	Died 9th day.
	P.10	"	Slight reaction—recovered.
III	F.1870	0.5	Died 5th day.
	F.1871	1.0	Remained normal.
	F.1872	1.0	" "
	F.1873	2.0	" "
	P.11	1.0	" "
	P.12	1.0	" "
	P.13	1.0	" "
	P.14	1.0	" "
	F.1897	Control	Died 4th day.
	F.1898	"	Severe reaction—recovered.
	P.15	"	Died 10th day.
	P.16	"	Slight reaction—recovered.

F = Fowl. P = Pigeon.

As these preliminary tests appeared encouraging it was decided to examine further the value of vaccine prepared on these lines. Pigeons being less susceptible than fowls to this virus, and as the disease in them tends to be protracted, they were not used again in this work.

Virus

The experiments here recorded have been carried out mainly with "Herts" and "Turkey" strains of virus. The "Herts" strain was isolated by Mr. N.

Dobson in 1934 from an outbreak of the disease in Hertfordshire; this outbreak was diagnosed by Professor T. Dalling. The "Turkey" strain was isolated by Mr. F. D. Asplin from a fowl infected by contact with naturally infected turkeys. One batch of vaccine was made with each of the following strains: "Matale" and "Ampitiya" strains obtained from Ceylon through the courtesy of Mr. M. Crawford, Commonwealth Bureau of Animal Health; one German strain obtained from Mr. Asplin; one strain of pigeon-passage virus—this was the "Herts" strain that had been passed 20 times through Pigeons. Sufficient work was carried out with these six strains to show that they were immunologically identical. It may well be that further search might reveal strains of higher immunogenic value than the "Herts" and "Turkey" strains used in these experiments. For testing for immunity a new batch of allantoic fluid was titrated on fowls every four months and stored at —60° C. As regards plurality of viruses, it can only be said that no evidence of this has so far been found, despite the numerous investigations carried out in this country, the United States, and elsewhere.

Susceptibility of Experimental Fowls

The experimental fowls, with the exception of three small groups bought from a nearby farm, were supplied by Mr. N. Dobson, from the laboratory flock of R.I.R. fowls, which is kept in strict isolation, and which has never been infected with any major disease. These fowls proved highly susceptible to the various strains of Newcastle virus used. The fowls were taken from the laboratory flock to an outlying farm, where all vaccinations were carried out. Throughout the period of isolation on this farm a considerable number of unvaccinated control fowls were kept in the pens as a means of detection of Newcastle virus should it have been inadvertently introduced; these unvaccinated fowls were used later as virus controls. The precautions taken against the introduction of infection were successful; the control fowls remained normal, and subsequently proved susceptible when inoculated with virus. When vaccinated fowls were due for virus tests they were brought to the Institute and put in separate cages in a wired-in, bird-proof enclosure.

Preparation of Vaccine

Ten-day-old fertile hens' eggs are inoculated with virus into the allantoic cavity; and those embryos that die within 72 hours and have extensive hæmorrhages of the feather follicles are used for vaccine production. The egg yolk and allantoic fluid are included in the vaccine; the albumen is discarded, although it was found in later tests that its inclusion did not have any injurious effects on the vaccine. The yolk and allantoic fluid of each egg is tested separately for sterility; the allantoic fluid is tested also for the presence of virus by the hæmagglutination test. The pooled contents of the selected eggs are passed through a colloid mill and filtered through sterile muslin. The virus content of each batch is estimated by the inoculation of eggs. Should the virus content be less than 10^{-6} the batch is discarded; this has occurred on

only one occasion. To each 800 g. of egg contents is added 200 c.c. of crystal violet-ethylene glycol (1 : 400). The mixture is incubated for 36 days at 37.5° C. On removal from the incubator, a sample of the vaccine is diluted, so that the dye content is not greater than 0.00005 per cent, and tested for sterility.

Safety Test of Vaccines

Two chickens, six-eight weeks old, are given 1 c.c. of vaccine intramuscularly, and two adult fowls 5 c.c. All should remain normal. Ten days after vaccination, an H.I. test is made on the serum of each adult fowl, and this should not show more than 40 units of inhibition.

Inactivation of Virus in Vaccine during Incubation

In the early stages of this work it was considered that incubation of vaccine for 21 days at 37° C. would not only inactivate the virus, but would leave a fair margin of safety. This procedure was based on that used in the preparation of swine fever vaccine, in which the virus, in a mixture of defibrinated blood, ethylene glycol and crystal violet, is inactivated by about the fifth day of incubation, and incubation is continued until the 14th day to eliminate the danger of virus surviving. Preliminary tests, in which Newcastle vaccine, after varying periods of incubation, was inoculated into fertile eggs, appeared to support this view. In tests of a number of batches of vaccine, prepared with different strains of virus, it was found that the virus was inactivated about the 11th to 13th day (Table II, page 144).

In these tests on eggs, with six batches of vaccine, prepared with different strains of virus (indistinguishable immunologically), it was found that the inactivation point of the virus lay between the 11th and the 13th day of incubation (37.5° C.). In the light of further experience, it became evident that the virus could remain alive for a longer period in vaccine than indicated by the egg test.

In view of this experience, comparative tests were made with four vaccines on fowls, adults and chickens aged 10-12 weeks, and on fertile eggs. The adult fowls were given 5 c.c. of vaccine and the chickens 1 c.c. Three ten-day-old fertile eggs were inoculated in the allantoic cavity with 0.1 c.c. of vaccine. The vaccines consisted of two batches prepared with egg passage "Herts" and "Turkey" virus, respectively; and two batches prepared with the same strains after each had been passed three times through fowls and then back to eggs. This was done because these viruses had been continuously passaged over a number of years through eggs, and also to determine if passage of virus through fowls would prolong its period of viability in vaccine.

A sample was taken every alternate day, from the 11th to the 36th day, from each vaccine, and tested on two fowls and on three eggs. Table III gives the results from the 12th to the 28th day with one vaccine (egg-passage "Herts" virus).

TABLE II.

Vaccine	Virus strain in vaccine	Concentration of virus in vaccine	Tested after incubation—Days	Eggs (3) inoculated (0.1 c.c.) with vaccine	Results—Virus
H.I.	Herts	10^{-7}	9	3/3*	Alive
			10	3/3	„
			11	3/3	„
			12	1/3	„
			13	0/3	Dead
			14	0/3	„
T.I.	Turkey	10^{-8}	9	3/3	Alive
			10	3/3	„
			11	1/3	„
			12	0/3	Dead
			13	0/3	„
P.I.	Pigeon	10^{-7}	9	3/3	Alive
			10	3/3	„
			11	3/3	„
			12	3/3	„
			13	0/3	Dead
			14	0/3	„
M.I.	Matale	10^{-6}	9	3/3	Alive
			10	2/3	„
			11	2/3	„
			12	1/3	„
			13	0/3	Dead
			14	0/3	„
A.I.	Ampitiya	10^{-6}	9	3/3	Alive
			10	2/3	„
			11	1/3	„
			12	0/3	Dead
			13	0/3	„
G.I.	German	10^{-7}	9	3/3	Alive
			10	1/3	„
			11	0/3	Dead
			12	0/3	„

*The numerator indicates the number dead, and the denominator indicates the number inoculated.

TABLE III.

Fowl Number	Vaccine Incubated Days	Dose of Vaccine c.c.	Results	H.I. Test Units	Viability on Eggs (3)
3742	12	1.0	D. 4th day		+++
3741		1.0	D. 5th day		
3749	14	5.0	D. 4th day		+
3761		1.0	D. 5th day		
3603	16	5.0	D. 5th day		—
3768		5.0	D. 5th day		
3783	18	1.0	D. 5th day		—
3786		1.0	D. 5th day		
3586	20	5.0	D. 5th day		—
3569		5.0	D. 5th day		
3789	22	1.0	D. 7th day		—
3788		1.0	D. 7th day		
3605	24	5.0	D. 7th day		N.D.
3563		5.0	D. 7th day		
3905	26	1.0	Remained normal	40	„
3907		1.0	„ „	40	
3615	28	5.0	„ „	10	„
3993		5.0	„ „	10	

N.D. = not done.

Fowls inoculated on the 30th and on the 32nd day remained normal.

The test on fowls proved that the virus could remain active until the 24th day of incubation, whereas the eggs were negative from the 14th day. The greater accuracy of the fowl test depended, presumably, to some extent on the larger inoculum, the dose given to chicken being ten times greater, and that for adult fowls 50 times greater, than used for eggs.

The other batches of vaccine gave the following results:—

"Herts" fowl-passage virus: fowls positive up to 20th day; eggs negative 14th day.

"Turkey" egg-passage virus: fowls positive up to 20th day; eggs negative 14th day.

"Turkey" fowl-passage virus: fowls positive up to 18th day; eggs negative 14th day.

The failure to demonstrate active virus in eggs after the fourteenth day was probably the result of an interference phenomenon similar to that described

by Henle and Henle (1944). They showed that inactivated influenza virus interfered under certain conditions in the allantoic sac with the propagation of active virus. In our tests the progressively increasing amounts of inactivated virus in the daily inoculum of vaccine presumably interfered with the propagation of the residual active virus. From this it would appear that the inoculation of eggs for determining the inactivation point of virus in the vaccine is unreliable. As the inactivation point, judged by the inoculation of fowls, appeared to be between the 18th and 24th day, it was decided, as a routine measure, to incubate vaccine for 36 days, so as to allow a reasonable margin of safety.

Route of Inoculation

In this test the immunity response to intramuscular inoculation of vaccine was compared with that of the intraperitoneal route; the subcutaneous route is unsatisfactory in fowls because of their thin skin. The fowls were given 100 M.L.D. of virus 49 days after vaccination (Table IV).

TABLE IV.

Fowl Number	Route of Inoculation	Dose of vaccine c.c.	Results
2492		1.0	Remained normal
2499	I/M	0.75	" "
2508	2 doses at interval	0.5	" "
2511	of 10 days	0.25	" "
2500		0.1	D. 8th day
2513		1.0	Remained normal
2496	I/M	0.75	" "
2506	One dose	0.5	D. 5th day
2495		0.25	Remained normal
2494		0.1	" "
2509		1.0	D. 6th day
2501	I/p	0.75	D. 6th day
2512		0.5	D. 5th day
2507		0.25	D. 10th day
2498		0.1	D. 6th day
2487	Virus control	—	D. 5th day
2502	" "	—	D. 5th day

I/M = intramuscular. I/p = intraperitoneal.

There was little difference in the results obtained between one and two doses of vaccine given intramuscularly; two doses would be impracticable under field conditions because of the extra labour and expense involved. The intraperitoneal route was apparently unsatisfactory for this type of vaccine. One c.c. of vaccine inoculated into the breast muscle was therefore adopted as the routine method of immunisation. To study the effect of vaccine on the muscular tissue, a series of fowls was vaccinated (1 c.c.) and one killed and examined

each 24 hours. In the fowl examined 24 hours after injection there were a few small hæmorrhages in the muscle and a faint trace of dye. In those examined from the 48th hour onwards there were neither lesions nor staining of the tissues.

By Inhalation

An attempt was made to immunise chickens by allowing them to inhale vaporised vaccine. Twelve chickens, 10 weeks old, were placed in a glass booth (3′ x 2′ x 2′ 6″) and allowed to inhale for one hour on three successive days vaccine passed through an "atomiser." A second group of chickens to act as controls were inoculated (0.5 c.c.) with vaccine from the same batch. Thirty days later both groups were given 50 M.L.D. of virus. The chickens treated with the "atomised" vaccine died, whereas those inoculated remained normal.

Establishment of Immunity

To determine the interval after vaccination when immunity is established, 12 cockerels, 14 weeks of age, were each injected with 1 c.c. vaccine. On the second day after vaccination, and on each succeeding day, one vaccinated and one control fowl were inoculated with 100 M.L.D. virus. Eleven of the twelve controls died in an average period of 4-8 days; the remaining control showed a severe reaction and recovered (Table V).

TABLE V.

Number of Fowl	Dose of Vaccine	Virus (100 M.L.D.) Days after vaccination	Results
2307	1 c.c.	2	Severe reaction—recovered
2275	,,	3	Died 5th day
2306	,,	4	Remained normal
2304	,,	5	Severe reaction—recovered
2298	,,	6	Died 5th day
2302	,,	7	Remained normal
2299	,,	8	,, ,,
2303	,,	9	,, ,,
2301	,,	10	,, ,,
2309	,,	11	Died 7th day
2308	,,	13	Remained normal
2300	,,	14	,, ,,

It would appear from these results that immunity is established about the seventh day after vaccination.

Immunity Tests

Vaccinated fowls were tested for immunity either by:—

(1) Direct contact with affected fowls.

(2) Intramuscular inoculation of virus.

Contact Test

In these tests, two vaccinated fowls, one susceptible fowl and one fowl inoculated with virus, were put in a cage (2′ x 2′ x 2′). The virus fowl usually

showed evidence of infection by the fourth or fifth day, and passed it on to the susceptible in-contact fowl; in this way the vaccinated fowls were subjected to continuous, severe infection. To keep up infection the fæces were allowed to accumulate in the cages, and it is possible that the bad hygienic conditions created may, at times, have adversely influenced the resistance of the fowls, as the results were sometimes erratic. This cage test was eventually discarded and a similar test tried in a fold-unit (4′ x 12′), but here, despite the fact that the virus (" Herts ") inoculated fowls always died, in no instance did infection pass to the susceptible in-contact controls. But when the egg-passage virus, which had been used up to this point, was replaced by a field virus that had not been subjected to egg-passage, the susceptible in-contacts contracted infection. It would appear that the " Herts " virus, as a result of egg-passage, had lost a considerable degree of its invasiveness, but retained its virulence by inoculation.

TABLE VI

" Turkey " Vaccine tested against Inoculation of Virus.

Tested interval after vaccination Days	Fowl Number	Dose of vaccine	Results
60	2001	1 c.c.	Remained normal
	2002	"	" "
	2003	"	" "
	2004	"	Slight reaction—recovered
	2005	"	Remained normal
	2006	"	" "
	2007	"	" "
	2008	"	" "
	2009	"	" "
	2037	Virus control	Died 6th day
	2033	" "	Died 8th day
125	2025	1 c.c.	Remained normal
	2024	"	" "
	1461	Virus control	Severe reaction—recovered
	731	" "	Died 6th day
154	2028	1 c.c.	Remained normal
	2028	"	" "
	1930	Virus control	Died 6th day
182	2011	1 c.c.	Killed—fowl pox
	2027	"	Remained normal
	2717	Virus control	Died 6th day
205	2014	1 c.c.	Remained normal
	2029	"	" "
	3007	Virus control	Died 8th day
239	2022	1 c.c.	Remained normal
	2034	Virus control	Died 6th day

The fold-unit test was later discontinued, although it appeared to be fairly reliable, because of the risk of spreading infection to other fowls on the premises.

Inoculation of Virus

Immunity tests were finally restricted to the intramuscular inoculation of virus (allantoic fluid); 100 M.L.D. were given to adult fowls and 50 M.L.D. to chickens.

Duration of Immunity

Vaccines prepared with either " Herts " or " Turkey " strains of virus were used in the duration of immunity experiments. The vaccinated fowls were tested at varying intervals, against either the inoculation of virus (100 M.L.D.) or against continuous contact infection.

The results are given in Tables VI-VIII..

TABLE VII. *" Turkey" Vaccine against Contact Infection in Cages.*

Tested interval after vaccination Days	Fowl Number	Dose of Vaccine	Results
125	2013	1 c.c.	Remained normal
	2017	"	" "
	2577	Virus control	Died 12th day
	2575	Contact control	Severe reaction—recovered
154	2021	1 c.c.	Remained normal
	2026	"	Slight reaction—recovered
	1930	Virus control	Died 6th day
	2046	Contact control	Died 10th day
182	2023	1 c.c.	Remained normal
	2010	"	" "
	2038	Virus control	Died 9th day
	2035	Contact control	Died 9th day
205	2019	1 c.c.	Remained normal
	2016	"	" "
	3007	Virus control	Died 8th day
	3009	Contact control	Slight reaction—recovered
239	2039	1 c.c.	Remained normal
	2020	"	" "
	2032	Virus control	Died 24th day
	2018	Contact control	Died 26th day

Summary of " Turkey " Vaccine

Sixteen out of 17 (94 per cent) vaccinated fowls were protected when tested at intervals varying from 60 to 239 days against the inoculation of virus. Seven out of eight (87 per cent) virus controls died, and in one there was a severe reaction. All ten vaccinated fowls resisted continuous contact infection; the five virus control fowls died; and three of five in-contact controls died; one reacted severely, and in one there was a slight reaction.

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TABLE VIII. *"Herts" Vaccine Tested against the Inoculation of Virus.*

Tested intervals after vaccination Days	Fowl Number	Dose of Vaccine	Results
120	2723	1 c.c.	Remained normal
	2705	"	" "
	3130	Virus control	Died 5th day
157	2712	1 c.c.	Remained normal
	2703	"	Died 6th day
	2719	"	Remained normal
	2722	"	" "
	3198	Virus control	Died 7th day
187	2726	1 c.c.	Died 4th day
	2702	"	Remained normal
	2713	"	" "
	2720	"	" "
	3556	Virus control	Died 5th day
216	2715	1 c.c.	Remained normal
	2721	"	" "
	2711	"	" "
	2707	"	Died 6th day
	3562	Virus control	Died 5th day
249	2714	1 c.c.	Remained normal
	2708	"	" "
	2701	"	" "
	2718	"	" "
	2453	Virus control	Died 7th day
281	2716	1 c.c.	Remained normal
	2717	"	" "
	2724	"	" "
	2740	"	" "
	2728	Virus controls	Severe reaction—paralysed
	2731	"	Died 20th day
	2746	"	Died 6th day
296	2736	1 c.c.	Remained normal
	2732	"	" "
	2741	"	" "
	2750	"	" "
	2745	Virus control	Died 6th day
335	2748	1 c.c.	Remained normal
	2753	"	" "
	2754	"	" "
	2730	"	" "
	4224	Virus control	Died 9th day
	4217	" "	Severe reaction—paralysed
365	2737	1 c.c.	Remained normal
	2744	"	" "
	2710	"	" "
	2747	"	" "
	2751	"	" "
	2734	"	" "
	2725	Virus control	Died 4th day
	2745	" "	Died 5th day
	2704	" "	Died 8th day

"Herts" Vaccine tested against contact infection in a fold-unit

In view of our experience that egg passage "Herts" virus was highly virulent when inoculated intramuscularly into fowls, but had lost its invasiveness by contact, a field strain of virus was obtained for use in this test. Eight vaccinated fowls, two unvaccinated controls and three fowls inoculated with spleen suspension (2 c.c.), from a fowl that had died of the field virus, were put in a fold-unit (4′ x 12′) (Table IX).

TABLE IX.

Tested interval after vaccination Days	Fowl Number	Doses of Vaccine c.c.	Results
266	2158	1.0	Remained normal
	2159	1.0	" "
	2340	1.0	Died 7th day
	2330	1.0	Remained normal
247	2331	1.0	" "
	2334	1.0	" "
	2319	1.0	" "
	2336	1.0	" "
	3364	Contact control	Died 17th day
	3365	" "	Died 19th day
Controls	3366	Virus fowl	Died 9th day
	3367	" "	Died 10th day
	3368	" "	Died 8th day

Summary of "Herts" Vaccine

Thirty-three out of 36 fowls (91 per cent) tested at varying intervals from 120 to 365 days were protected against the inoculation of virus. Seven out of eight fowls (87 per cent) resisted continuous contact infection with a "field" virus when tested at intervals of 247 to 266 days after vaccination.

Duration of Immunity. Vaccination of Young Chickens

This experiment was carried out to determine the duration of immunity in chickens vaccinated at an early age; previous experiments were made on chickens 14 to 16 weeks of age. A group of chickens, seven weeks of age, were vaccinated and tested for immunity at intervals varying from 106 to 365 days by the inoculation of 100 M.L.D. of virus (Table X).

It would appear from these results that a durable immunity can be conferred on chickens vaccinated at seven weeks of age.

The Response of chickens of different ages to vaccination

As there appeared to be evidence in preliminary work that it was more difficult to immunise chickens of six weeks of age than those of eight or nine weeks, a comparative test was carried out. Five groups representing chickens of 6, 7, 8, 9 and 10 weeks of age, respectively, were vaccinated; each group con-

TABLE X.

Tested after vaccination Days	Fowl Number	Dose of Vaccine c.c.	Results
	2832	1.0	Remained normal
106	2844	1.0	" "
	Control—2130	—	Died 5th day
	2851	1.0	Remained normal
192	2849	1.0	" "
	Control—2836	—	Died 5th day
	2843	1.0	Remained normal
267	2846	1.0	" "
	Control—2835	—	Died 7th day
	2850	1.0	Remained normal
321	2847	1.0	" "
	Control—2834	—	Died 9th day
	2845	1.0	Remained normal
365	2841	1.0	Died 11th day
	Control—2839	—	Died 5th day

sisted of five chickens, three of which were given 0.5 c.c. of vaccine and two 1 c.c. Twenty-two days later these chickens, with five controls, were given 0.5 c.c. (50 M.L.D.) of virus (Table XI, page 153).

It would appear from these results that there is no appreciable difference in the immunity response of chickens varying in age from six to nine weeks. Twenty-two of the 25 chickens were solidly immune when tested 22 days after vaccination. All the virus control chickens died.

Immunisation of Turkeys

A small test was carried out to see whether the vaccine protected turkeys. Two groups of turkeys, aged four months and two months, respectively, were given varying doses of vaccine and inoculated 30 days later with 100 M.L.D. of virus. A group of fowls was included to verify the potency of the vaccine. (Table XII, page 154).

It would not be safe to draw conclusions from the vaccination of the four-month-old group, as one of the two virus controls remained normal. The three vaccinated birds in the two-month-old group proved solidly immune. This small test suggests that the vaccine can be used for the protection of turkeys.

Effect of Storage on Vaccine

The keeping qualities of the vaccine is a point of practical importance, as in the event of production on any considerable scale it would be essential to know the period during which it would retain its potency in cold storage (2-4 degrees C.).

Experiment No. 1

A batch of vaccine was tested for potency after 160 days in cold storage. Six cockerels, five months of age, were inoculated with 0.5 c.c. 1 c.c., and 2 c.c. of vaccine; two fowls were used for each dose. Twenty-one days later these fowls, with two controls, were given 100 M.L.D. of virus. The vaccinated fowls remained normal, and the two controls died on the sixth day.

Experiment II

Nine batches of vaccine that had been in cold storage for periods varying from 452 to 88 days were tested for potency. Each vaccine was tested in varying doses on four chickens, 16 weeks old; all vaccines were injected on the

TABLE XI.

Age of chickens	Number	Dose of vaccine	Result
6 weeks	1796	0.5 c.c.	Remained normal
	1794	,,	,, ,,
	1811	,,	,, ,,
	2984	1.0 c.c.	,, ,,
	1817	,,	,, ,,
	1834	Control	Died 6th day
7 weeks	1280	0.5 c.c.	Remained normal
	1206	,,	,, ,,
	1203	,,	,, ,,
	1216	1.0 c.c.	,, ,,
	1330	,,	,, ,,
	1296	Control	Died 6th day
8 weeks	1310	0.5 c.c.	Remained normal
	1142	,,	,, ,,
	1471	,,	Died 6th day
	1248	1.0 c.c.	Remained normal
	1238	,,	,, ,,
	1244	Control	Died 4th day
9 weeks	2932	0.5 c.c.	Remained normal
	2884	,,	,, ,,
	2877	,,	,, ,,
	2880	1.0 c.c.	Died 5th day
	2900	,,	Remained normal
	2931	Control	Died 7th day
10 weeks	1479	0.5 c.c.	Remained normal
	1405	,,	,, ,,
	1351	,,	,, ,,
	1402	1.0 c.c.	,, ,,
	1313	,,	Died 5th day
	1178	Control	Died 5th day

TABLE XII.

	Number	Dose of Vaccine c.c.	Results
Turkeys four months of age	4031	2	Remained normal
	4034	4	" "
	4033	2	" "
	4035	4	" "
	4036	Virus control	Paralysed—killed in extremis
Turkeys two months of age	4032	" "	Remained normal
	4025	1	Remained normal
	4029	1	" "
	4028	1	Not tested—died Blackhead
	4030	2	Remained normal
	4027	Virus control	Died 8th day
	4026	" "	Died 4th day
Fowls four months of age	3281	1	Remained normal
	3286	2	" "
	3283	1	" "
	3288	2	" "
	2834	Virus control	Died 9th day
	4154	" "	Died 8th day

same day, and thirty-seven days later each chicken was given 100 M.L.D. of virus. The birds were kept in pens until inoculated with virus when they were transferred to individual cages. Three vaccines, aged 452, 431, and 355 days, respectively, conferred no protection, and are not included in Table XIII.

The test shows that vaccine retains its potency for at least 168 days when kept at 2-4 degrees C.

Experiment III

It would appear from the results obtained in Experiments I and II that vaccine stored at a temperature of 2-4 degrees C. retains its potency for at least five months. As this is an important practical point, as well as of some academic interest, some further work was carried out to verify these results.

A batch of vaccine (H.9) was tested on six occasions at monthly intervals from time of preparation, on chickens eleven weeks old; chickens from the same source, age and breed were used for each test. Twenty-five days after vaccination, each bird was given 50 M.L.D. of virus; the same batch of virus, stored at —60 degreees C., was used throughout the tests. The chickens in the group tested at 119 days were affected with coccidiosis, and two died before the inoculation of virus.

TABLE XIII.

Fowl Number	Vaccine	Age of vaccine Days	Dose of vaccine c.c.	Results
3239			1.0	Died 7th day
3218	H.3	272	0.75	Remained normal
3224			0.5	Died 8th day
3221			0.25	Lost
3207			1.0	Severe reaction—recovered
2794	H.A.	242	0.75	" " "
3214			0.5	Died 8th day
3212			0.25	Died 5th day
3232			1.0	Remained normal
3217	T.2	235	0.75	" "
3235			0.5	Killed fighting—before virus
3220			0.25	Died 9th day
3205			1.0	Remained normal
3213	H.5	173	0.75	" "
2793			0.5	" "
2795			0.25	Died 5th day
3422			1.0	Remained normal
3424	H.6	168	0.75	" "
3423			0.5	" "
3425			0.25	" "
3419			1.0	Remained normal
3418	H.9	88	0.75	" "
3420			0.5	" "
3421			0.25	" "
3240	Virus control		—	Died 5th day
3220	" "		—	Died 6th day

H = " Herts " virus. T = " Turkey " virus.

The results of the first two tests, i.e., immediately after preparation and at 30 days, are not given in Table XIV, as the vaccinated birds remained normal and the controls died.

TABLE XIV.

Tested after removal from incubator Days	Dose c.c.	Fowl Number	Results
60	1.0	3411	No reaction
		3414	„ „
	0.75	3402	„ „
		3401	„ „
	0.5	3403	„ „
		3404	Died 5th day
	0.25	3405	No reaction
		3406	„ „
	Control	3552	Died 4th day
90	1.0	3287	No reaction
		3388	„ „
	0.75	3389	„ „
		3390	„ „
	0.5	3391	„ „
		3392	„ „
	0.25	3393	Severe reaction—recovered
		3394	Died 6th day
	Control	3588	Died 5th day
119	1.0	3727	No reaction
		3728	„ „
	0.75	3734	„ „
		x3740	Died coccidiosis before virus inoculation
	0.5	3723	No reaction
		x3733	Died coccidiosis before virus inoculation
	0.25	3735	Severe reaction—recovered
		3724	Died 8th day
	Control	3726	Died 4th day
152	„	3737	Died 6th day
	1.0	3936	No reaction
		3937	„ „
	0.75	3938	„ „
		3939	Died 9th day
	0.5	3940	No reaction
		3941	„ „
	0.25	3942	Severe reaction—recovered
		3943	Moderate reaction—recovered
		3928	Died 6th day
	Controls	3929	Died 7th day

This experiment confirms the previous results that vaccine can be kept for five months in cold storage at 2-4 degrees C., but this should be regarded as the maximum period. One batch of vaccine, stored for 103 days at room temperature, was tested on fowls, and found to have decreased in potency. Three out of four vaccinated fowls survived the inoculation of virus, but all reacted.

Virus Neutralising Anti-bodies in Serum of Vaccinated Fowls

In preliminary tests it was found that 0.5 c.c. of pooled sera from vaccinated fowls neutralised 1,000 M.L.D. of virus; and that 0.5 c.c. pooled sera from normal fowls may neutralise 100 M.L.D.

Experiment I

To obtain further information, serum was obtained from 12 fowls, 33 days after vaccination, and before the test dose of virus; these fowls had been injected with vaccines that had been in cold storage (2-4 degrees C.) for periods ranging from 327 to 118 days. Nine fowls subsequently resisted the inoculation of virus, and three died. The sera were pooled in two lots:—

(a) From the nine vaccinated fowls that resisted infection.

(b) From the vaccinated fowls that died.

The pooled sera from the immune fowls was tested on five fowls (Group I) against falling dilutions of virus; the same procedure was followed with the pooled sera from the vaccinated fowls that had died (Group II); pooled sera from normal fowls and virus control fowls were included in the test (Groups III and IV). (Table XV.)

TABLE XV

		Fowls inoculated simultaneously			
Groups	Fowl Number	Pooled sera from	Virus (0.5 c.c.) M.L.D.	Serum c.c.	Results
I	2755	Immune fowls	100	0.5	Remained normal
	2758	" "	1,000	"	" "
	2761	" "	10,000	"	" "
	2757	" "	50,000	"	Died 7th day
	2768	" "	100,000	"	Died 7th day
II	2777	Non-immune fowls	100	"	Remained normal
	2767	" "	1,000	"	Died 9th day
	2766	" "	10,000	"	Died 7th day
	2770	" "	50,000	"	Died 7th day
	2760	" "	100,000	"	Died 5th day
III	2779	Normal fowls	100	"	Remained normal
	2769	" "	1,000	"	Died 6th day
	2765	" "	10,000	"	Died 12th day
IV	2764	—	100	Virus control	Died 12th day
	2762	—	1,000	" "	Died 7th day
	2771	—	10,000	" "	Died 5th day
	2763	—	50,000	" "	Died 5th day
	2756	—	100,000	" "	Died 5th day

The sera from the immune fowls neutralised 10,000 M.L.D. of virus; sera from the vaccinated fowls that died neutralised only 100 M.L.D.; normal sera neutralised 100 M.L.D.

Experiment II

A second experiment, similar to the first, but excluding sera from non-immune vaccinated fowls, was carried out with fowls immunised with vaccine prepared from " Turkey " virus. (Table XVI.)

TABLE XVI.

Fowl Number	Pooled sera from	Fowls inoculated simultaneously with Virus (0.5 c.c.) M.L.D.	Serum c.c.	Results
56	Immune fowls	100	0.5	Remained normal
91	,,	100	,,	,, ,,
67	,,	1,000	,,	,, ,,
75	,,	1,000	,,	,, ,,
58	,,	10,000	,,	,, ,,
65	,,	10,000	,,	Slight reaction
52	,,	100,000	,,	Died 5th day
59	,,	100,000	,,	Died 9th day
74	,,	1,000,000	,,	Died 5th day
89	,,	1,000,000	,,	Died 8th day
68	Normal fowls	100	,,	Died 9th day
84	,,	100	,,	Severe reaction—recovered
66	,,	1,000	,,	Died 9th day
80	,,	1,000	,,	Died 5th day
55	,,	10,000	,,	Died 6th day
76	,,	10,000	,,	Severe reaction—recovered
49	—	100	Virus control	Died 8th day
72	—	1,000	,,	Died 7th day
63	—	10,000	,,	Died 4th day
54	—	100,000	,,	Died 7th day

This experiment confirmed the previous results. In both experiments a preliminary H.I. test was made on the serum of each vaccinated fowl and none showed more than 10 units of inhibition.

Serum Neutralising Tests on Eggs

Serum neutralisation tests were carried out also on eggs, using the technique recommended by the Bureau of Animal Industry, Washington. The serum was obtained from seven of the immune fowls used in experiment No. 1; each fowl was injected (1 c.c.) with a different batch of old vaccine.

The serum from each fowl was tested separately. Three sera were negative. Four were positive, giving the following results:—

One serum neutralised 100,000 M.L.D. of virus.
Two sera „ 10,000 „ „ „
One serum „ 1,000 „ „ „

It is evident from these tests, carried out on fowls and eggs, that the serum of vaccinated fowls contain virus neutralising antibodies.

The Haemagglutination-inhibition reaction of Vaccinated Fowls

Fowls given 1 c.c. of vaccine intramuscularly do not give a positive H.I. reaction; in over 90 per cent of fowls the reaction was not greater than 10

TABLE XVII.

Fowl Number	Vaccine	Dose of vaccine c.c.	Results
2491		1.0	Remained normal
2517	+ Crystal violet	0.75	„ „
2530		0.5	„ „
2486		0.25	Died 6th day
2529		1.0	Died 5th day
2520	— Crystal violet	0.75	„ „ „
2527		0.5	„ „ „
2548		0.25	„ „ „
2519	Virus control	—	Died 5th day
2525	„ „	—	Died 6th day

units of inhibition, and in the remainder it varied between 20 and 40 units. In the majority of fowls receiving a 5 c.c. dose it varied between 10 and 20 units; none has been higher than 40 units. This is a considerable advantage, as it follows that the use of vaccine in the field would not interfere to any appreciable extent with the H.I. reaction for the detection of natural infection.

Brandly and his co-workers (1947), who carried out extensive investigations on the diagnostic procedure for Newcastle disease, state:—

" It is, nevertheless, impossible to identify the " antibodies " associated with hæmagglutination-inhibition, virus neutralisation, and specific refractivity to infection as a single entity." The evidence obtained here supports their view.

Crystal Violet

Crystal violet as an attenuating agent for the virus of swine fever was introduced in 1934 by the late Dr. Marion Dorset, Bureau of Animal Industry, Washington. He devoted much time to the preparation and testing of blood vaccines inactivated with different chemicals, but with the exception of those containing crystal violet all failed to give consistent results. The addition of this dye, either alone or in combination with phenol, to swine fever infected blood gave most encouraging results. It was found that the bactericidal action

of the dye varied with the pH of the vaccine, so to make it slightly more alkaline, Tilley (1939) recommended the addition of a small amount of dibasic sodium phosphate. The bactericidal action of the crystal violet - sodium phosphate mixture was, however, weak, and the vaccine gave a heavy precipitate on standing. Modifications were tried, and finally the dibasic sodium phosphate was replaced by either glycerine or ethylene glycol (Millar, 1943) with satisfactory results. We have, as already mentioned, followed this procedure in preparing a vaccine with Newcastle virus. In tests made on fertile eggs with vaccines prepared with and without crystal violet it was found that the virus in both products was inactivated at approximately the same time, but immunity tests made on fowls with these vaccines yielded markedly different results. A batch of vaccine was divided into two parts, to one part of which was added crystal violet in the usual amount. These vaccines were tested on two groups of cockerels, aged about four months. Twenty-five days later the vaccinated fowls with controls were given 100 M.L.D. of virus. (Table XVII.)

The vaccine containing crystal violet gave a reasonably good result, whereas the vaccine without crystal violet failed to confer protection. This experiment was repeated with another batch of vaccine and similar results were obtained. The rôle played by crystal violet is not clear, for in a vaccine containing ethylene glycol the weak bacteriostatic or bactericidal action of the dye would appear unnecessary. Neither can it be claimed that it is essential as an attenuating agent, as the virus in both vaccines was inactivated at about the same time. The mechanism of the crystal violet effect, in which inactivation of the virus is achieved without impairing the antigenic properties, is unknown.

Discussion

An inactivated tissue vaccine against Newcastle disease is described. It is prepared from finely minced chick embryos suspended in crystal violet-ethylene glycol; glycerine can be used in place of ethylene glycol. Most of the vaccine has been prepared from either " Herts " or " Turkey " strains of virus. Nothing was previously known of the immunogenic value of these strains; they were used because they were available at the Institute. Both strains proved satisfactory, but it may be that further search would reveal others of higher immunogenic value. Twenty-two batches of vaccine have been prepared, and twenty of these were of satisfactory potency. One batch prepared with a living, avirulent strain of virus failed to confer protection; this strain, when used unmodified, caused no symptoms in fowls and conferred a solid immunity. The immunogenic value of the strain of virus used in the production of vaccine is probably of cardinal importance. A point that requires examination is the suitability for vaccine production of virus that has been propagated over a long period on eggs. The " Herts " egg-passage virus has apparently lost its invasiveness for fowls by contact; it might be found advantageous to pass the virus occasionally through fowls. Immunity is established about seven days after vaccination. The duration of immunity has not been fully estimated, but it persists for at least twelve months. The vaccine does not cause any reaction,

paralysis or check in growth. Numerous tests have proved that vaccinated fowls are incapable of transmitting infection to susceptible in-contacts. It can be used safely on chicks from six weeks of age; there was some evidence that ethylene glycol was slightly toxic for chicks under one month of age; this point was not, however, fully examined. In one small test on turkeys the vaccine gave satisfactory results. The vaccine gives rise to virus neutralising antibodies, but does not induce a positive H.I. reaction—which is a considerable advantage, as it will not interfere with the use of the H.I. test for the detection of naturally infected fowls. No controlled tests were made to determine the effect of vaccination on egg production, but from casual observation it did not appear to have any adverse influence. A fact of considerable practical importance is that the vaccine retains its potency for about five months in cold storage (2-4 degrees C.); this should be regarded as the maximum period.

Comparative tests on eggs and fowls to determine the point of inactivation of the virus in vaccine during incubation showed that the egg test was the less reliable; the failure of the egg test was probably the result of interference in the allantoic sac between inactivated virus and the propagation of the residual active virus. The greater accuracy of the fowl test depended, no doubt, to some extent on the larger inoculum used.

Fowl Plague

Preliminary experiments have been carried out with an inactivated chick-embryo vaccine against fowl plague; this has been prepared similarly to the vaccine against Newcastle disease here described. The plague vaccine conferred a solid immunity against 100 M.L.D. of plague virus twenty-one days after vaccination. This result is of considerable interest, as general experience is that it is difficult to immunise against plague, and no method has yet been found sufficiently reliable to justify use in the field.

Conclusion

An inactivated chick embryo tissue vaccine against Newcastle disease is described.

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STUDIES ON EQUINE PURPURA HÆMORRHAGICA
Article No. 5—Some Biochemical Aspects

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THE four previous papers in this series have been concerned with (1) the earlier literature on equine purpura hæmorrhagica (Biggers and Ingram, 1948); (2) the clinical observations on the cases we have investigated (Bennett and King, 1948); (3) the morbid anatomy and histopathology of these cases (King, 1949); and (4) the hæmatological findings (Biggers, Ingram and Murray, 1949). In this paper an account is given of the biochemical findings.

Introduction

The literature dealing with the biochemical aspects of the condition in horses known as purpura hæmorrhagica is very scanty. Beyond a few scattered references to odd semi-quantitative experiments on the bloods and urines of affected animals, there appear to be few, if any, detailed analyses, either of single samples, or of serials from the same animal, over a period of time during which the symptoms were manifest.

Hutyra and Marek (1917), quoting Smith, state that protein substances and fat are higher than in normal blood, whereas fibrin, water and iron are present in diminished quantities.

More recently, two French investigators, Bellocq and Louf (1943), have stated that in a chronic case of purpura they obtained a mucilaginous urine, but with normal density and yellow in colour; further, they were unable to find any albumin, sugar, urobilin, urinary indoxyl or porphyrin in the urine.

With such sparse information available, it was planned to start investigations *de novo,* and, if possible, to correlate the biochemical investigations with those of a more physiological nature, described in a previous paper in this series (Biggers, *et al.,* 1949). The statement that a possible cause of the syndrome might be a malfunctioning of the blood-clotting processes stimulated the inclusion of a general survey of the blood proteins and inorganic ions, with special reference to calcium and fibrin. That a quantitative as well as a qualitative survey of the urines should have been included is obvious, since so many normal metabolic processes can be checked by reference to their products in the urine, and it seemed feasible that some hitherto unidentified disturbances might be traced through the urinary constituents. It was not possible to investigate fully more than two cases, and the two actually studied, horses C and F (see Bennett and King, 1948), illustrated, on the one hand (C), a very prolonged manifestation of the symptoms which resulted ultimately in the destruction of the animal, and, on the other (F), a horse which was affected for a relatively short time and then recovered. Blood samples were collected at regular intervals, these being the same samples as those used for the

physiological and histological blood picture studies, which are recorded in a previous paper of this series (Biggers, *et al.*, 1949). Urines were collected at less frequent intervals, this being due, in part at any rate, to the difficulty in seizing the opportunities when micturition occurred.

RESULTS

These are divided into two sections (I and II), the first dealing with the studies made on the bloods, and the second on the urines. Where possible, graphs have been used in place of tables, in order that the chronological sequence of events may be made quite clear. Quite obviously, a true picture cannot be given where there were considerable gaps between samples. This was the case with the blood samples of horse F, and the urines of both horses.

Section I—Blood

(i) PROTEINS AND NON-PROTEIN-NITROGEN.

General Methods.

The first four samples from horse C were analysed relatively completely, giving total blood nitrogen, and plasma values for N.P.N, fibrin, globulin and albumin (Table 1).

TABLE 1

Blood analyses of the first four samples of horse C

(*All quantities are expressed in gms./100 ml., except N.P.N., which is in mgms./100 ml.*)

Day (Feb.).	Total Blood Nitrogen.	Total Plasma Protein.	Fibrin.	Globulin.	Albumin.	N.P.N.
1	2.6	6.6	0.45	3.7	2.4	22
3	2.75	5.7	0.45	3.8	1.4	19
4	3.2	5.5	0.50	3.6	1.45	—
5	—	—	0.55	3.7	—	27

After this it was decided to analyse all the remaining ten samples for albumin, as these figures were likely to be the most significant if capillary permeability was abnormal. When this was completed, the globulin and N.P.N. figures were obtained for these remaining ten samples.

Samples from horse F were analysed, along with the latter ten from horse C, for albumin, globulin and N.P.N. only.

A sample from a normal horse was analysed for albumin, globulin, N.P.N. and fibrin for comparison, and these figures are quoted alongside others obtained from the literature (Table 2, page 164).

Finally, some œdematous and peritoneal fluids were obtained from horses C and D, after destruction of the animals. These were examined qualitatively and quantitatively.

Plasma figures for albumin, globulin, fibrin and N.P.N. were determined, after fractionation, by micro-Kjeldahl estimations, as described by Harrison (1947). The total blood nitrogen was obtained by diluting 2 ml. of whole blood to 50 ml. with physiological saline, and using 1 ml. of this fluid for the Kjeldahl digestion. In most plasma samples slight hæmolysis had occurred, and it was

found that the resulting hæmoglobin appeared in the albumin fraction; it was therefore necessary to make allowance by deducting values obtained for hæmoglobin (Van-Slyke method) from the "total" albumin figures.

This paper was submitted for publication in October, 1949, since references have been made to it with this date.

TABLE 2

Comparative figures for horse plasma proteins

(*Units as for Table* 1.)

Authority.	Albumin.	Globulin.	Fibrin.	N.P.N.
Wladasch (1936)	3·43	3·417	—	74
Roby and Jones (1947)	3·77	3.21	0.84	—
Kehar, *et al.* (1940)	4.641	3.656	—	—
Damboviceanu (1929)	2.42	5.03	—	—
Sakaguchi, *et al.* (1918)	3·32	3·34	—	31·9
Derivaux (1942)	3·39	4·34	—	—
Stefanopoulo and Codounis (1930) ...	2.72	5·354	—	—
Courmont, *et al.* (1930)	3·35	2·73	—	23
Demski (1941)	4.22	3·76	0.34	—
Rajcsanyi (1941)	2.22	6.08	—	—
Author's normals	2.56	3·51	0.2	24·5
Mean	*3.28*	*4.04*	—	—

Discussion of the results for proteins and N.P.N.

From Table 1, it can be seen that the fibrin values were fairly constant, but that the third value (February 3) for total blood nitrogen was fairly high, as would be expected from the hæmoconcentration which appeared to commence at this time (see Biggers, *et al.*, 1949). The albumin, globulin and N.P.N. figures from this table are included in Graphs 1a and 1b.

(A) *Albumin—Horse C.*

Graph 1a gives these results. The significant fact is that the value on the first day, February 1, 1947, was 2.4 gms./100 ml., and at this time there was no marked œdema in the horse; whereas on the third day the value was 1.4 gms./100 ml., a fall of 1.0 gm./100 ml., and gross œdema was observable. From this date onwards the value remained approximately constant at about 1.4 gms./100 ml. Normal values for the horse appear to be at least 2.2 gms./100 ml., or usually higher (Table 2), thus indicating that the value obtained, 1.4 gms./100 ml., was definitely subnormal, which could be explained by a loss of albumin into the œdema fluid.

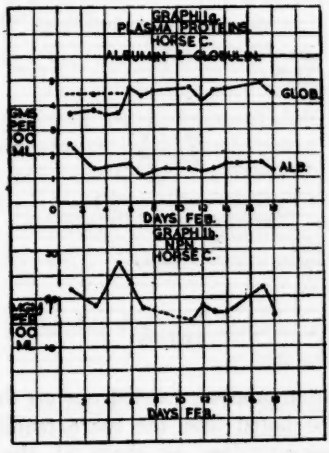
(B) *Globulin—Horse C.*

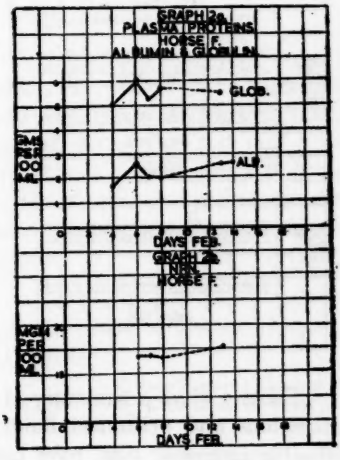
The globulin curve is also shown on Graph 1a; the first four figures are fairly constant, and the next nine are also constant at a higher value. This change was due to a delay of two or three weeks between estimations four and five, whilst others were being completed. This was confirmed by repeating the second estimation afterwards, giving a value in line with the later ones, as shown

by the dotted line on the graph. Thus, the globulin figure was in all probability constant throughout the period at about 33.7 gms./100 ml., and this is a possible normal value (see Table 2).

(C) *N.P.N.—Horse C.*

Graph 1b shows the results obtained. The variations are within normal limits, and since it is one of the most variable factors in the blood, even in the normal course of events, not many conclusions can be drawn. It is considered, therefore, that the only significant fact is that the N.P.N. figures are within normal range.





(D) *Albumin, Globulin and N.P.N.—Horse F.*

Graph 2a shows the corresponding variations in the first two of these constituents for this horse, and Graph 2b the N.P.N values. As only four or five determinations were obtained, the results are not very significant, especially as there is a gap of five days between two of the readings.

Points worth noting are:—

(a) The N.P.N. values are normal, as with horse C.

(b) The albumin shows a general increase from subnormal back to normal, as would be expected from the fact that this horse made a complete recovery.

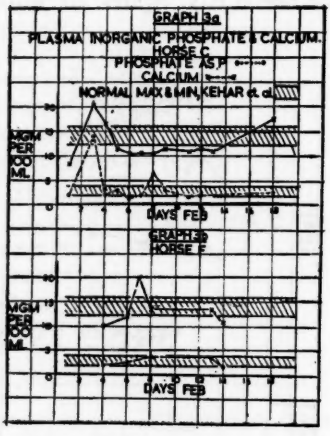
(c) The globulin values are higher than with horse C. This is, however, a possible variation within the normal range, since the figures recorded in the literature show wide variation (see Table 2).

(E) *The Œdema and Peritoneal Fluids.*

The œdema fluid gave only an extremely weak benzidine reaction, indicating a very small quantity of hæmoglobin. It had a normal N.P.N. value of 22 mgms./100 ml., but, on the other hand, it had a total protein content of about 1 gm./100 ml., which consisted almost entirely of albumin. No fibrin

could be detected by the usual methods, and only traces of globulin. These facts provide substantiating evidence for increased capillary permeability in this syndrome.

Finally, a considerable quantity of fluid was obtained from the peritoneal cavity of horse D. This fluid was pale pink in colour (hæmoglobin), but gave only a moderately strong benzidine reaction. It appeared to contain less than 0.1 gm./100 ml. of hæmoglobin, and this probably resulted during the dissection.



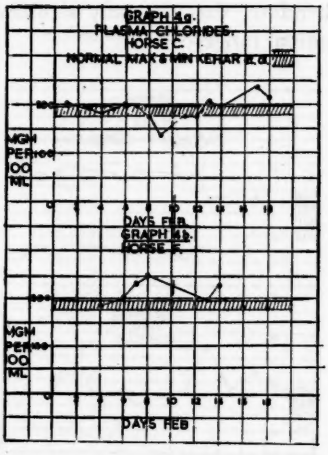


Table 3 gives a summary of the quantitative analyses carried out on the œdematous and peritoneal fluids. It will be noted that the more readily diffusible inorganic ions, have comparable concentrations to those of these substances in the plasma.

TABLE 3

Analyses of œdematous and peritoneal fluids—horse C

(All quantities are expressed in mgm./100 ml., except protein, which is in gms./100 ml.)

Fluid.	Total Protein.	Albumin.	Globulin.	Fibrin.	Calcium.	Inorganic Phosphate (as P).	Chloride (as Cl.).
Œdema ...	1.0	*	trace	nil	10.2	3.4	198
Peritoneal ...	+		trace	nil	8.9	3.0	200

* *Probably about 0.9 gm./100 ml. or more.*

(ii) INORGANIC CONSTITUENTS.

General.

Three only were investigated, viz., calcium, inorganic phosphate and chloride. These are shown for both horses on Graphs 3a, 3b, 4a and 4b, respectively. In view of some divergence of normal values, as summarised by Kehar, Singh and Rao (1940), and supplemented by their own analyses, cross-hatched areas have been included on the graphs, to represent those maxima

and minima of normals reported by these authors for a series of different horses. The figures given in Table 4 summarise the results obtained by Kehar, *et al.*, together with other figures quoted by them.

Discussion of the results for inorganic constituents.

(A) *Calcium—Horse C.*

A marked concentration (see Graph 3a) occurred between February 1 and 3, and this reached the very abnormal figure of 20 mgms./100 ml. The peak then fell off towards the fifth day, when the level was maintained between 10 and 12 mgms./100 ml. (probably a normal figure—see other figures in Table 4) until February 13, after which an isolated high value of 17.5 mgms./100 ml. was found.

(B) *Phosphate—Horse C.*

This followed a very similar trend to calcium (see Graph 3a); a maximum appearing about the third day (14 mgms./100 ml.), dropping back thereafter to more or less normal figures—between 1.5 and 3.0 mgms./100 ml. The sudden rise to approximately 65 mgms./100 ml. about February 8, is doubtless due to a fairly large degree of hæmolysis in this sample, and some attendant hydrolysis of organic phosphates. It will be noted that there was no correspondingly high calcium concentration at this period.

TABLE 4

Calcium, Inorganic Phosphate and Chloride in the blood of Normal Horses

(*All values in mgms./100 ml.*)

Constituent.	Max. and Min. (Kehar, et al.).	Other Figures Quoted by Kehar, et al. (1940).
Calcium	16—12	11.2, 11.1
Inorganic Phosphate (as P)	3.8—2.12	3.5, 6.4
Chloride (as NaCl) ...	320—290 (Av. 306)	451,* 290.4

* *This figure is mgm./100 ml. of* BLOOD, *not serum.*

(C) *Calcium and Inorganic Phosphate—Horse F.*

The values obtained are shown in Graph 3b. Analogous results to those of horse C were obtained, but the maximum value for calcium did not correspond with a maximum for inorganic phosphate as with horse C, and the phosphate figures for horse F appear to lie within the normal range. Since this horse recovered, the last two readings can probably be accepted as normal, and they show reasonable agreement with those obtained by Kehar, *et al.* (loc. cit.). The last figures for horse F were: Calcium, 11.0 mgms./100 ml.; inorganic phosphate, 1.9 mgms./100 ml.

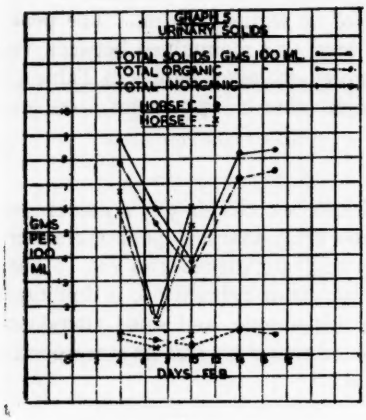
(D) *Chlorides—Horse C.*

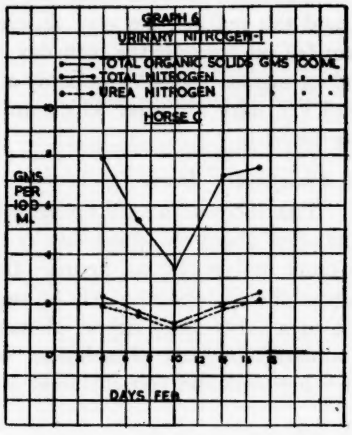
The chloride figures are shown in Graph 4a. There is some loss of chlorides between February 8 and 10, and this may possibly be correlated with a slight rise in urinary chlorides during the same period (see Graph 8). This was followed by a fairly steady rise, which similarly corresponds with a diminution of urinary chlorides. It is interesting to note that the concentration of chlorides

in the œdema and peritoneal fluids is comparable with that of the plasma, as would be expected by a constant balancing up of these ions.

(E) *Chlorides—Horse F.*

Little can be learnt from the values obtained, as there are not enough for accurate chronological observations.





Section II—Urine

Discussion of Results.

(A) *General physical characteristics and qualitative analysis.*

The majority of the urines examined were thick and viscous, and varied in colour from dark brown (concentrated) to pale yellow (dilute). The concentrated urines all possessed a deposit, which, when examined chemically and microscopically, proved to consist mainly of urates. Table 5 gives a summary of the main physical properties.

TABLE 5

Physical Properties of the Urines

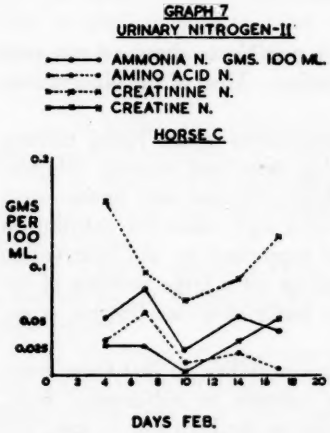
Date Withdrawn.	Horse.	Specific Gravity.	pH.	Colour.
February 4	F	1.030	7.8	brown
February 7	F	1.014	5.2	yellow-brown
February 10	F	1.034	7.0	brown
February 4	C	1.036	7.2	brown
February 7	C	1.033	6.1	light-brown
February 10	C	1.018	5.2	yellow
February 14	C	1.031	5.2	yellow-brown
February 17	C	1.033	5.7	light-brown
February 4	D*	1.034	8.0	brown

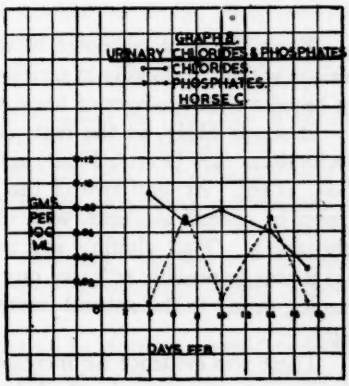
* *This urine was not from horses C or F. It was withdrawn from the bladder of horse D after destruction.*

In general, a low specific gravity seems to be correlated with a drop of pH, but beyond this the only point of interest is the rather low pH of some samples.

(B) *The distribution of dissolved solids.*

These are shown in Graphs 5 and 6. Variations ranging from 3.8 to 8.8 gms./100 ml. were recorded for horse C, and 1.6 to 6.7 gms./100 ml. for horse F. Of the total solids, the great bulk proved to be organic in nature, and most of this consisted of urea. In general, the variations in total dissolved solids were mirrored by those of dissolved organic solids. On the other hand, the inorganic constituents exhibited a marked degree of constancy in concentration, for even with the very great dilution recorded in both urines on February 7 and 10, respectively, there was only a very slight drop in the inorganic constituents. These were 0.4-1.00 gm./100 ml. for horse C, and 0.3-0.9 gm./100 ml. for horse F.





(C) *Nitrogen partition—Horse C only.*

The fluctuations recorded for total and organic solids, are reflected in the nitrogen partition shown in Graphs 6 and 7. The three main points of interest are :—

(a) The slight rise in concentration of ammonia N. and amino-acid N. from February 4 to 7, followed by a sharp drop and then a rise.

(b) The sharp drop of urinary creatinine from February 4 to 10, with a subsequent rise, but without any maximum during this period.

(c) The steady increase of urinary creatine from February 10 onwards, indicating a possibility of muscular dystrophy, which was subsequently verified by pathological examination (see King, 1949).

(D) *Hippuric Acid.*

No serial investigations were made for this substance, but three representative samples of urine were analysed, giving results as follows:—

C	...	...	0.05 gm./100 ml.
F	...	...	0.104 gm./100 ml.
D	...	...	0.30 gm./100 ml.

(E) *Inorganic Constituents.*

Phosphate and chloride were estimated in horse C only, and these results are shown on Graph 8. There is little to be learnt from the erratic phosphate figures, but the chlorides show a very marked falling off in concentration, possibly correlated, as previously stated, with a corresponding increase in the concentration of plasma chlorides.

(F) *Pathological Constituents.*

Only in horse D was any blood found in the urine, and the test was not very strong. Otherwise, no abnormal constituents (sugar, albumin, bile salts, ketones, blood) were found in any of the urines examined.

Discussion

From the facts presented in this study, it is possible to draw certain conclusions without indulging in unwarranted speculation. Most of the observations are based on the analyses of horse C.

It seems likely, in the first place, that disturbances of the blood clotting mechanisms can be ruled out, since the clotting time was normal (Biggers, *et al.*, 1949). At least two of the factors involved—calcium and fibrin—were present in normal amounts, with the exception of a high value for calcium on February 3. That clotting was normal is also supported by the observation that it was impossible to carry out fibrin estimations on a large number of the samples, owing to the natural formation of large, bulky clots (see Biggers, *et al.*, 1949).

There was evidence for increased capillary permeability, in that there was: (i) a permanent drop in the plasma albumin content to sub-normal level, and (ii) the protein content of the œdema fluid was on the high side (1 gm./100 ml.), when compared with œdema fluid of nutritional or nephrotic origin, containing, in man, 0.2 gm./100 ml. or less (Wells, 1925; Bodanski and Bodanski, 1940).

It is noteworthy, in connection with the blood analyses on samples from horse C, that maxima occurred in the calcium and phosphate content at the beginning of the period of hæmo-concentration (Biggers, *et al.*, 1949), whereas there was no increase of plasma chloride at this point. One possible explanation for this is that the capillary membranes, although exhibiting an increased permeability to proteins, were in some way inhibiting the passage through them of the more highly charged ions (calcium and phosphate). This point should not be over-emphasised, however, since the distribution of any charged particles will vary, as a result of the changing permeability of the membranes.

A later observation, which might be related to these unexpected concentrations of calcium and phosphate, concerns horse C; when this animal was

destroyed, and the bones were subjected to maceration to remove adhering tissues, the osseous tissue itself was also rapidly destroyed, thus indicating a very low inorganic content. It is possible that during life there was a mobilisation of calcium and phosphate from the bones. This could account for the high calcium and phosphate of the plasma on February 2 and 3.

From the urinary analyses carried out, it would appear that excretion and protein metabolism were normal. Moreover, with the exception of one post-mortem case, the absence of the more common pathological constituents points to a normal, or relatively normal, course in the mechanisms involved in the production of these substances.

The inorganic dissolved solids of the urines showed remarkably little variation. Any variation in the excretion of sodium, if due to malfunctioning of the adrenal cortex, would be compensated, in part at any rate, by a reciprocal variation in the excretion of potassium. Thus, even if we assume no variation in other inorganic constituents, any such changes would not be shown by the total inorganic solids in the urine. Moreover, the adrenals of horse C showed no gross abnormality on histological examination (see King, 1949). It is hoped that we shall be able to carry out a fuller examination of the distribution of inorganic ions, with cases of equine purpura hæmorrhagica in the future.

Summary

1. A preliminary biochemical study has been made on two cases of equine purpura hæmorrhagica, in which serial analyses have been carried out.

2. A leakage of albumin into the œdema fluid has been demonstrated, and in one case, where recovery occurred, re-establishment of plasma constituents to normal values.

3. Results indicate that there are differing degrees of capillary permeability, as evidenced by variations in the retention of certain plasma constituents.

4. The blood clotting mechanism appears to be normal, as evidenced by the plasma concentrations of calcium and fibrin, and also by the spontaneous formation of clots in a very large number of blood samples.

5. The findings of Smith, as reported by Hutyra and Marek (1917), cannot be fully substantiated; there is some agreement with Bellocq and Louf (1943), although the latter workers have not reported any serial or quantitative figures.

6. No pathological constituents were found in the urines of the two horses examined serially.

7. It has been concluded, as a result of the blood, and more particularly the urine, analyses ,that the protein metabolism at any rate is fairly normal; also, in view of the absence of reducing sugars and ketone bodies, there is no indication of abnormality of the carbohydrate and fat metabolic processes.

Acknowledgments

The authors wish to acknowledge their indebtedness to Messrs. J. D. Biggers, P. Scott-Dunn, and H. Mills for supplying material, to Professor E. C. Amoroso for many helpful discussions, and to Mr. H. A. Burgess for help in photographing the graphs.

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REVIEWS

DANISH FOREIGN OFFICE JOURNAL No. 4, 1949.

IN this recently published number an interesting and instructive article on foot-and-mouth disease is included: it is termed "Danish drive against foot-and-mouth disease—practical result of a new vaccine," and is written by Dr. Sven Schmidt, Head of the Foot-and-Mouth Disease Laboratories. The article traces the history of the vaccine now used—the adsorbed heated virus to which formalin is added—and relates in brief the method of preparation. Following the original work of Sven Schmidt and his colleagues in Denmark, the vaccine was tested out in cattle on the Island of Reims by Waldman in 1936 and was used on a large scale in Germany early in 1938. Following the reports of the success of the vaccine in Germany, vaccine was produced in Denmark on a factory scale, partly on the Island of Lindholm and partly at the State Serum Institute, Copenhagen. Figures are given on the use of the vaccine during the great foot-and-mouth disease epidemic in the late 1930's. In Germany, 40,676 cattle were vaccinated in 1938; in 1942, Waldman reported that 3,600,000 cattle had been vaccinated, that solid immunity developed in twelve to fourteen days and lasted for eight to twelve months; the percentage of immunity is reported as 99.5. In Denmark, between November, 1938, and December, 1940, 1,385,093 cattle in 71,542 herds were treated. In this period the disease had been diagnosed in 55,247 herds, but only in 506 herds in which vaccination had been carried out. This means that only 0.7 per cent of

vaccinated herds became infected, or only 0.05 per cent of herds in which there had been time for the development of full immunity following vaccination.

The maximum weekly production of vaccine in Denmark is about 3,000 litres, which is sufficient to vaccinate about 120,000 cattle. From Denmark vaccine is exported to various countries, and other countries have now set up production establishments on lines similar to those in Denmark.

Reference is also made to the work of Fraenkel in Amsterdam: he has succeeded in cultivating the virus outside the animal body, and vaccine can be produced from such virus. The use of irradiation of the virus by ultra-violet rays is also mentioned, a more potent vaccine being the result of this treatment. Traub's recent work in Germany on the cultivation of the virus in the chick embryo is also referred to.

FIRST AID HINTS FOR THE HORSE OWNER, by Lt.-Col. W. E. Lyon. New and revised edition, 1950. Price 10s. 6d. net. London: Collins, St. James Place.

THE average person who comes into contact with animals seems to acquire a desire to do a little "home doctoring." It does not appear to matter if such "doctoring" be necessary or not.

This book should do the horses good, for it confines its attention to first aid measures, definitely indicates when they may be usefully applied, and also the occasions when professional advice should be sought. We say that "it should do the horses good" for the reason that an owner who studies its pages intelligently will confine his amateur efforts to simple measures and diseases which call for first aid measures, and leave the more serious troubles to the professional man.

On behalf of the horse we can recommend the book to all horsemen.

KIND KILLING, compiled by F. Jean Vinter, Technical Secretary of U.F.A.W. Price 3d. London: The Universities Federation for Animal Welfare, 284, Regent's Park Road, Finchley, N.3.

DEATH is a reckoning that all living creatures must meet, and just as we hope that our own end may be "just a sleep and a forgetting," so would we have it with our animals. In our case the end may come because of accident, disease or age; in their case we have to add humane and economic reasons. Whatever the necessity, suffering in any shape or form cannot be condoned. A great deal of sentimental nonsense clouds the issue. The sentimentalist is apt to liken the destruction of an animal to the death of a human being, and he forgets that in the latter case the "terrors of death" are based mainly on a premeditation of events to come. In animals—and certainly when their death is by design—such a factor should play no part, or at the most be only momentary.

This small pamphlet should do good. The subject is approached on logical, scientific lines, but the humanitarian view is abundantly manifest throughout. The language used is simple, the instructions are precise, and all those who may have to undertake the task of ending a small animal's life, be they experienced or not, can profit by a perusal of this small but timely book.

INTRODUCTION TO CLINICAL DIAGNOSIS OF INTERNAL DISEASES AND SKIN DISEASES OF DOMESTIC ANIMALS, by Professor D. Wirth (formerly Director of the Medical Clinic of the Veterinary High School in Vienna) (1949). Price approx. 20s. Third edition. Vienna: Urban-Schwarzenberg.

THIS small book was first published in 1934 as a general introduction to clinical veterinary medicine for students at the Vienna Veterinary High School. The book was developed from notes given by Professor Wirth to his students over a period of some fifteen years, and it was at their wish that the printed notes were incorporated into a textbook. The present edition retains the characters of the first edition in size, scope and arrangement, but the text has been revised and a number of illustrations have been added and others improved.

The aim of the book is to provide a general introduction to clinical examination and diagnosis, and thus to give a sound foundation on which students can build their subsequent clinical experience during their years of clinical study.

There are thirty-six short chapters which deal in order with the procedures adopted in making a clinical examination of a sick animal. The book stresses the need for thoroughness and order in clinical study, to investigate each area systematically, and to miss nothing. The importance of an adequate case history, methods of restraint, mechanical aids to diagnosis, the state of nutrition of the patient, percussion and auscultation, examination of blood and urine, sending in material to a laboratory—these are some of the subjects dealt with.

Professor Wirth's book will continue to serve well the needs of students in Vienna and in the German veterinary schools. For students in other countries some small sections may be unnecessary and there will be aspects which require elaboration, e.g., tuberculin testing in cattle.

HORSE KEEPER'S ENCYCLOPEDIA, by W. H. Walter. Price 5s. net. Kingswood, Surrey: Andrew George Elliot (Right Way Books), Glade House.

BEFORE the advent of the motor car, when the horse was supreme, the average user of that animal did not worry much about the technical details of horsemastership. He was content to leave those matters to the groom. Nowadays this is not the case, for, although the numbers of horses have declined, we find that the majority of horsemen and -women, and not excepting the children, are deeply interested in all that may concern their equine friends.

For economic reasons many of these people have "to do" their mounts themselves, and they show a great desire to enhance their knowledge.

To them this little book will prove to be interesting and instructive, and one to which they can turn in a moment to settle any point in dispute.

It has excellent illustrations, line drawings and photographs. Of the latter, those which depict the British breeds are very good. There are twenty-two chapters and two appendices. There is no great waste of words, and the format is good and itemised in a manner that makes reference easy.

THE COMPARATIVE ANATOMY AND PHYSIOLOGY OF THE LARYNX, by V. E. Negus, M.S., F.R.C.S. Price 30s. net. William Heinemann Medical Books, Ltd. 1949.

THE author, surgeon to the Ear, Nose and Throat Department of King's College Hospital, has gathered together in just over two hundred pages a wealth of detail on the larynx of a very wide range of animals. In the introduction, he points out that erroneous interpretations may be made if the organ is considered as being evolved for phonation instead of as a means of preventing the entrance of foreign bodies into the pulmonary air tracts.

The first chapter deals with the evolution of the larynx as it appears in the fish up to its more elaborate structure in mammals, and the succeeding six chapters with the acquirement of additional functions. Examples quoted of modifications are for olfaction in the main associated with the epiglottis, nasopharynx and soft palate, for respiration the ability to alter the size of the larynx, and for deglutition the formation of lateral food channels.

The anatomy of the air passages is dealt with from a comparative aspect, and it is convincingly argued that the air sacs of various animals are developed for rebreathing and not for voice purposes. Three chapters are devoted to the production and use of sound in animals, the author concluding that the mode of life, rather than anatomical structure, governs these accomplishments. Man's intelligence has enabled him to make full use of his versatile larynx, and this, in turn, has advanced his intelligence. This is compared to the cat, which also has a wide range of pitch that could, if intelligently used, be a voice of considerable merit, "but as it is the rising and falling cadences of domestic cats are hideous." The final chapter is concerned with the anatomy of the human larynx, and the three appendices are lectures the author has delivered to learned bodies.

The script is a pleasure to read; the numerous illustrations and beautiful line drawings make the book, as is claimed, a pictorial guide to parts of the Museum of the Royal College of Surgeons. The publishers are to be congratulated on their production, for it puts a finishing touch to a fine work.

R.N.S.

BRITISH FARM STOCK, by the Earl of Portsmouth. Price 5s. London: Collins.

EVERYTHING the Earl of Portsmouth writes commands not only the attention, but the deep interest of all who have to do with animal husbandry. He has now added to his great reputation by the polished and instructive essay

he has published under the title of "British Farm Stock," in the "Britain in Picture" Series. It is a delightful philosophical and highly factual survey of the history of stockbreeding from the earliest days, illustrated in the charming way that all the many volumes in this Series are. How deeply the pattern of our countryside has been influenced by our stockbreeding methods, how much the pattern of our lives has been changed and our national character formed by our livestock is not often realised: still less is it appreciated how well and truly our rude forefathers developed the basic stocks which were ready to hand when the time came for scientific methods of breeding to be developed. Lord Portsmouth sets out three main improvements needed to-day. First and foremost, the education of the milk producer and the stockman in good animal management. Secondly, the universal use of improved sires, and, thirdly, the elimination of most of our diseases.

As is well known, he believes firmly in, and has consistently advocated, a whole and ecological outlook, and nowhere has he with greater succinctness or more telling persuasiveness demonstrated how landscape, climate, soil and environment have not only been responsible for the development of our national character, but have, and still are, conditioning the evolution of our livestock, and he therefore states once again his belief in the undoubted need for scientific farmers of to-day to recreate intensive mixed farming on a scale never before realised. But in the end it is the happiness and well-being of mankind we all seek, and so Lord Portsmouth concludes with these words: "The stockman's is the most responsible of all farm work. It is necessary to the nation's soul as well as its stomach, for the greatest crop the land can yield is desirable and happy human beings." Was ever great truth more simply or more clearly set down that all who run may read and learn.

CORRESPONDENCE

To the Editor of THE BRITISH VETERINARY JOURNAL

SIR,—In your March issue Dr. J. R. M. Innes gives an excellent review of recent publications on demyelinating diseases. As Dr. Innes has misinterpreted my remarks on post-distemper encephalitis, in a talk I gave to the S.W.V.S. in February of last year and reported in the *Veterinary Record* in June, I ask for the use of your columns to make the matter clear.

I suggested that the development of encephalitis following distemper inoculation might be similar in character to post-vaccination encephalitis occurring in humans, and I mentioned that I understood such an encephalitis (a disseminating encephalomyelitis) does occasionally follow rabies injections in dogs. I have never had an opportunity of having brain material from "post-distemper injection encephalitis" examined histologically and my remarks which follow refer to the pathology which is usually described as being found in post-vaccination encephalitis in humans.

Dr. Innes did not misinterpret my view when I suggested that encephalitis has often followed attempts at distemper prophylaxis. I am perhaps not justified in calling this condition encephalitis, but clinically it is indistinguishable from the other encephalopathes, fits (hysterical and epileptic), paralysis amaurosis, chorea, etc. Dr. Innes himself mentions that the early reports on demyelinating encephalitis in dogs following rabies vaccination were very few and merely recorded the occurrence of clinical paralysis, and this in spite of the fact that millions of dogs must have been inoculated against rabies and the first paper to mention demyelination was not until Von Mócsy's in 1938. I have often been surprised at the absence of reports on post-distemper vaccination illness in veterinary literature. As a practitioner, however, I can understand the diffidence of a veterinary surgeon in reporting the loss of, say, fourteen puppies out of twenty inoculated three weeks previously. Such a report would be a gratuitous offer of the type of evidence which would substantiate any claim for compensation in a Court of Law by the aggrieved owner. I have known this proportion of illness subsequent to distemper inoculation, but in this particular case I unfortunately have no records of post-mortem examinations and it is quite possible the illness had nothing whatever to do with demyelinating disease.

Between 1929 and 1949 I inoculated over 3,500 dogs against distemper, using vaccine virus method, and in this number more than 1 per cent developed illness within fourteen days of the administration of the vaccine. Many of these cases were no doubt due to intracurrent infection, but there was some evidence in many cases to rule this out and the illness conformed to that found in post-vaccination encephalitis. I am not alone in having such cases; I know many veterinary surgeons in this country have had similar trouble. In the Glasgow Veterinary School we are carrying out investigations into virus diseases in dogs, and we have not so far had an opportunity of investigating the above type of

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material; but we would welcome the opportunity if any practitioner is unfortunate enough to run into such cases.

I can understand the reluctance of Dr. Innes to accept the view that vaccine made from spleen and lacking in nervous tissue should be responsible for initiating demyelinating diseases, but the only satisfactory way to rule out its possible occurrence is to carry out extensive histopathological examination and I am prepared to keep an open mind on the nature of the illness until we have had an opportunity of examining such material.

Yours faithfully,

WILLIAM L. WEIPERS.

Glasgow,
April 6, 1950.

NOTICE

THE Crookes Laboratories announce the introduction of their Calamine Lotion (Veterinary) as from April 1. The use of this preparation, which is a stable suspension of semi-colloidal nature, is indicated in all non-parasitic skin conditions, otitis and in all pruritic skin conditions.

It is available in the following packs (all prices subject to the usual discounts): 8 oz. bottles, 2s. 6d. (purchase tax 7d.); 80 oz. bottles, 22s. (dispensing pack—exempt from purchase tax).

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THE BRITISH VETERINARY JOURNAL, with which is incorporated THE VETERINARY JOURNAL, is published monthly, and copy for advertisements should be in the hands of the publishers—Baillière, Tindall and Cox—not later than the 20th of the preceding month, if proof is required. Tel.: Temple Bar 3386.

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